*MECHANISM OF PROFLAVIN MUTAGENESIS**

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Abstract.—The mutagenic action of proflavin on bacteriophage T4 is greater in the presence of defective T4 ligase than in the presence of normal T4 ligase. This suggests that the persistence of single-strand breaks in DNA enhances proflavin mutagenesis.

The original hypothesis of Brenner, Barnett, Crick, and Orgel,[1] which states that acridine dyes cause additions and deletions of base pairs in DNA, is now well substantiated. The classical genetic experiments of Crick, Barnett, Brenner, and Watts-Tobin[2] demonstrated that proflavin-induced mutations shift the reading frame of the *r*IIB cistron of bacteriophage T4. This has been confirmed by means of biochemical experiments by Streisinger and his colleagues (Okada *et al.*,[3] Terzaghi *et al.*,[4] Streisinger *et al.*[5]), who studied proflavin-induced mutations in the lysozyme (*e*) gene of T4.

Various models have been proposed to explain how acridine dyes such as proflavin cause additions and deletions in the nucleotide sequence of DNA (see Brenner *et al.*,[1] Lerman,[6] Drake,[7] Streisinger *et al.*[5]). For some of these models, breaks in the DNA are obligatory, and for some they are not. Streisinger *et al.*[5] have suggested that single-strand breaks in DNA near regions of repeating base sequence would allow mispairing of the repeating sequence. This mispairing would be stabilized by acridines, and DNA synthesis contiguous to the mispaired region would result in the addition or deletion of bases.

To test whether single-strand breaks enhance proflavin mutagenesis, we have made use of a phage strain with a temperature-sensitive mutation in gene 30. Phage carrying the mutation *ts*B20 have been shown by Fareed and Richardson[8] to produce a temperature-sensitive ligase at 37°C. We postulate that if only defective mutant ligase is present in infected bacteria, single-strand breaks would not be repaired efficiently; proflavin could then induce mutations at a frequency higher than that which occurs when normal wild-type ligase is available to repair the breaks.

We have taken the reversion of FC55, a T4 *r*IIB cistron mutant of + sign (Crick *et al.*[2]), as a measure of proflavin mutagenesis. We have compared the proflavin-induced reversion frequency at 37°C for FC55 (which produces normal ligase) with the reversion frequency at 37°C for the double mutant strain *ts*B20 FC55 (which produces defective ligase). Table 1 summarizes our results.

Our results show that the frequency of reversion at the FC55 site is an order of magnitude higher in the strain with the defective ligase (*ts*B20 FC55) than it is in the strain with normal ligase (FC55).

In experiments in which bacteria were infected simultaneously with both FC55 and *ts*B20 FC55 phage particles, we observed low reversion frequencies similar to those in infections with FC55 alone.

TABLE 1. *Effect of tsB20 on proflavin mutagenesis.*

Expt.	r → r⁺ Net Reversion Frequency × 10^{-8} FC55	*ts*B20 FC55	FC55 and *ts*B20 FC55	Increase (no. of times) in reversion frequency due to *ts*B20
1	1.0	9.9	...	9.9
2	6.2	72.0	8.9	11.6
3	4.1	38.9	...	9.5
4	2.9	37.4	...	12.9
5	1.0	28.3	...	28.3
6	1.7	16.5	...	9.7
7	2.7	50.1	4.2	18.6
8	1.2	28.4	...	23.7
9	4.2	48.1	4.9	11.5
10	8.7	97.6	8.3	11.2
11	1.0	9.1	...	9.1
12	6.6	100.6	12.3	15.2
13	4.3	41.1	...	9.6
			Average:	13.9
			(S.D. =	5.8)

Proflavin mutagenesis was carried out according to the method of Barnett *et al.*[9] For each mutagenesis experiment a control experiment was performed without proflavin.

Net reversion frequency = reversion frequency with proflavin − reversion frequency without proflavin. (The average reversion frequency without proflavin was 2% of the reversion frequency with proflavin.)

We conclude that the significantly higher proflavin-induced reversion frequency observed in the infection with the *ts*B20 FC55 strain is due to the accumulation of single-strand breaks in DNA. In mixed infections with FC55 and *ts*B20 FC55 phage, the breaks are repaired by the normal ligase, and hence the proflavin-induced mutation frequency is about the same as in infections with FC55 alone. The double infection results rule out the possibility that the *ts*B20 ligase itself is mutagenic in the presence of proflavin.

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